LOCALIZED INDUCTION OF CORTICAL SPREADING DEPRESSION WAVES IN BRAIN SLICES USING MICROFLUIDIC INJECTION

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ABSTRACT

Advanced *in vitro* experimental tools for locally stimulating and probing brain slices can greatly facilitate systematic studies of neuronal phenomena and rapid drug screening. A major challenge in developing such tools is to achieve precise spatial and temporal control of local chemical environments of brain slices while maintaining their viability. This paper reports an improved design of a brain slice perfusion chamber with integrated microfluidic ports for controlled local introduction and confinement of soluble chemicals on slice surfaces. As a concrete example of potential clinical relevance, we present a systematic characterization of the area-dependent threshold concentration of potassium ions for the induction of cortical spreading depression (CSD) waves.

KEYWORDS: Cortical spreading depression, Brain slice chamber, Localized stimulation, Microfluidics

INTRODUCTION

Brain slices harvested directly from animal models represent a useful neurophysiological preparation for studying many aspects of cellular and network functions in brain [1]. The ability to chemically control the local extracellular environment of brain slices is of critical importance in drug screening and understanding spatial-temporally varying neuronal phenomena such as seizures and cortical spreading depression (CSD) [2].

Microfluidic devices have recently emerged as an elegant add-on to traditional brain slice perfusion chambers to introduce chemicals into extracellular spaces. Previously reported microfluidic devices [3-7], however, have been limited in that they cannot confine chemical plumes in both lateral directions, they may introduce undesired mechanical trauma, or they may otherwise compromise the viability of brain slices.

In this paper, we use an improved design of integrated microfluidic ports reported in [8] for controlled spatial delivery of soluble chemicals onto slice surfaces. We apply this device to quantify the threshold concentration of potassium ions necessary for the induction of CSD waves. Past studies [9] suggested that there is a minimum volume of tissue that must be exposed to an elevated concentration of potassium ions for the onset of CSD waves. Inability to precisely define and maintain a chemical plume, however, has so far precluded quantitative determination of the threshold ion concentration and its dependence on stimulation areas. By employing chemical injection ports of different sizes, we systematically characterize the area-dependence of the threshold potassium ion concentration for chemically induced CSD waves.

DEVICE DESIGN

Our slice perfusion chamber uses an array of PDMS (polydimethylsiloxane) micro-posts to maintain a steady flow of an artificial cerebrospinal fluid (aCSF) solution. Selected posts are replaced with fluidic ports, each consisting of one injection port paired with several suction ports to realize a localized plume on each port [8]. Our microfluidic port and experimental setup are schematically shown in Figure 1.

In our previous port design, the injection port was recessed with respect to the suction ports. Although this design enabled an effective spatial confinement of an injected chemical, it was susceptible to port clogging in the presence of fluctuations in injection or suction pressure. Combined numerical simulation and flow/concentration visualization experiments are used to identify relative heights of the port walls that can prevent such clogging without compromising the ability to confine chemical plumes. The diameter of the fluidic ports is varied from 400 to 1000 μm to cover physiologically relevant tissue volumes in rodent brains.

EXPERIMENTAL

Two separate pumps are used for independent control of injection and suction flows, respectively. The brain slice is imaged using a 4x objective (UPlanSApo, Olympus America, Melville NY) coupled to a high sensitivity camera (Watec 902H2 Ultimate, Tsuruoka, Japan) through a filter cube (excitation 470+/-20nm, dichroic 505nm, emission>515nm; Chroma Technology) for epifluorescence imaging. Plume size is extracted from the fluorescent images as illustrated in Figure 2.

CSD is induced by delivering a potassium-rich ACSF solution containing a certain molarity of KCl substituted for an equimolar NaCl. A trace amount of fluorescein (<0.1%) is added for flow visualization.

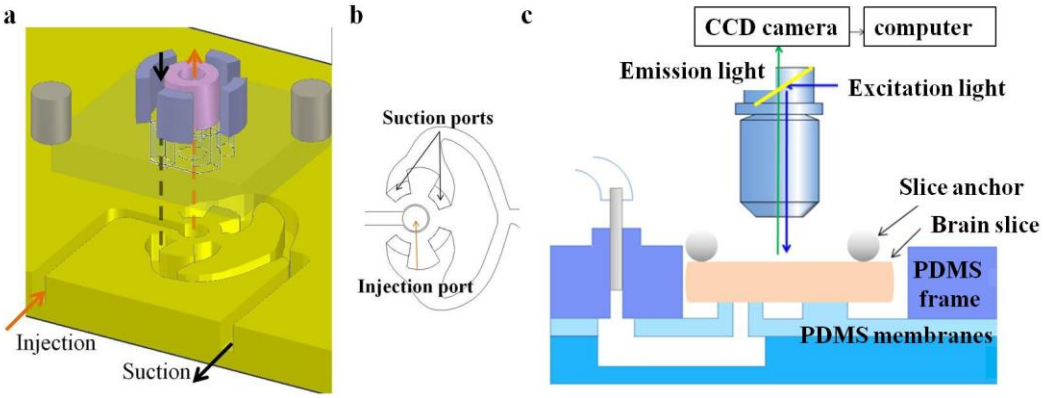

Figure 1: (a) Schematics of the fluid injection and suction ports. (b)The top view of the fluidic ports. (c) Experimental setup for CSD induction experiments. Simultaneous white light and fluorescence imaging are used to detect CSD waves and also quantify the spatial extent of chemical stimulations.

For each injection event, a successful CSD induction is checked from the presence of a dark wave initiating from the origin of stimulation, usually within 20 seconds after the stimulation reaches the slice. The injection channel is then flushed with an oxygenated normal aCSF and the slice is left to recover for ten minutes. A fresh potassium-rich aCSF solution where the potassium concentration is increased (or decreased) by 5 or 10 mM is then applied through the same port. The stepping process continues until CSD is elicited (or stops being elicited). The same procedure is repeated for fluidic ports of different sizes to determine the corresponding threshold potassium concentration.

RESULTS AND DISCUSSION

Concentric CSD waves were successfully induced (Figure 3) by locally injecting potassium-rich ACSF solutions onto cortical layers of rodent brain slices. Figure 4 show the measured threshold potassium concentration as a function of the port size. The threshold concentration decreases rapidly with the port size for port radii below 300 μm.

The "minimum" threshold concentration corresponding to stimulation areas over 1 mm^2 is approximately 18 mM. This value is consistent with values independently determined by bathing entire slices in potassium-rich ACSF solutions. The threshold potassium concentrations observed in the present study range from high levels one may find under traumatic conditions to low levels encountered due to normal fluctuations in the physiological settings.

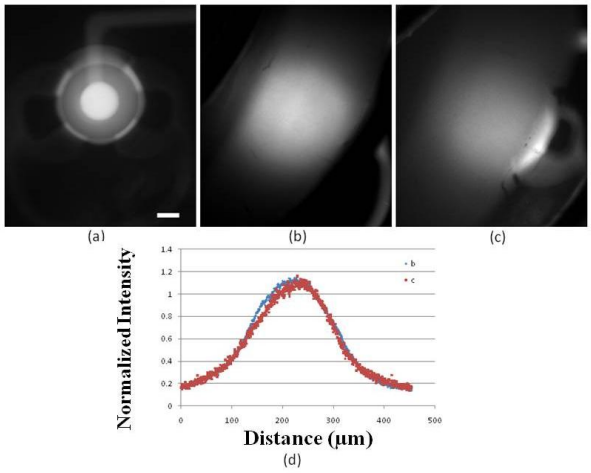

Figure 2: Fluorescent images showing the plumes of ACSF solutions mixed with fluorescein without a brain slice on top (a) and with a slice on top (b, c). (d) shows the normalized fluorescence intensity profiles for two independent experiments to demonstrate repeatability.

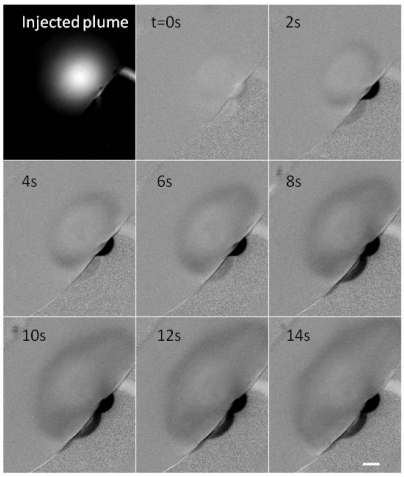

Figure 3: Temporal montage of a CSD wave initiated by local injection of a 40 mM K^+ solution (t = 0 to 14seconds). The upper left inset shows the location of the injected plume observed through a brain slice using the fluorescent microscope. Scale bar: 100μm.

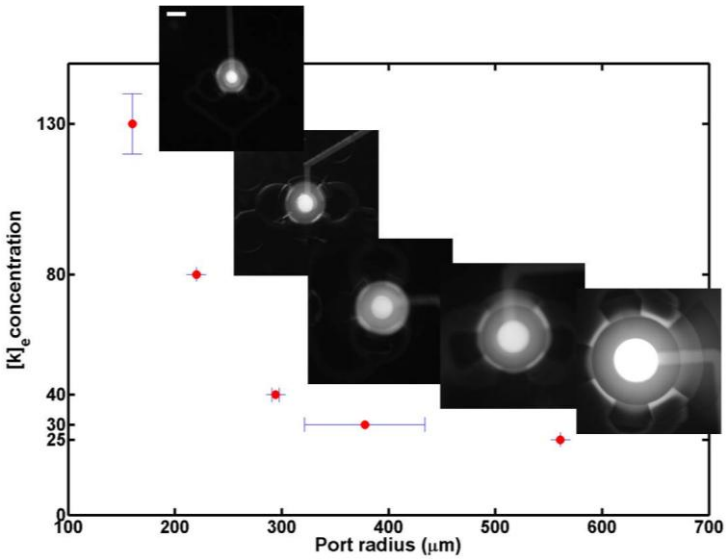

Figure 4: Threshold potassium ion concentration for the onset of a CSD wave as a function of port size.

CONCLUSION

An improved multilayer microfluidic device with the ability to both improve perfusion (and hence slice viability) and locally inject chemicals in a precisely controlled manner has been demonstrated. The device has been successfully applied to characterize the threshold of chemically induced cortical spreading depression. Our work establishes an experimental tool to characterize brain slices under localized chemical stimulations and demonstrates how they can be used to improve our understanding of neuro-physiological events and ultimately their effective mitigation.

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